CTT Media

Ingredient	Proportion	Quantity
Bacto-Casitone	1%	10 g
Tris-HCl tampon (pH 7.6)	10 mM	1.21 g
$MgSO_4$	8 mM	0.96 g/10 mL (PM=120.366)
Phosphate Tampon (pH 7.6) • $KH_2 PO_4$ • $KH PO_4$	1mM	1 L

Electroporation Protocol for M. xantus (Based on Kashefi and Hartzell)

*Every procedure is performed at room temperature unless otherwise noted

1. Grow an overnight culture at 32ºC in Casitone-Yeast Extract (CYE) to an optical density at 600 nm (OD_{600}) of 0.5-1.0
2. For each electroporation, pellet 1.5 mL of culture at 15 000 rcf in a microcentrifugue for 4 min. Aspirate off medium.
3. Was the cells by resuspending in 1 mL of sterile distilled deionized water (ddH_2O).
4. Pellet again (repeat step 2). Aspirate the water (pellet may become loose)
5. Repeat washing with sterile ddH_2O 2 or 3 times.
6. Resuspend the cells in 40 uL of sterile ddH_2O.
7. Add 1 to 5 uL of salt free DNA to the cell suspension and mix by vortexing
    a. 100-300 ng of replicative plasmid or plasmid w/ phage attachment site
    b. 0.5-1.5 ug of plasmid for integration by homologous recombination
    c. 3-5 ug of genomic DNA
8. Transfer cells and DNa to an electroporation cuvette with a 1 mm gap and perform electroporation with:
    a. Resistance at 400 ohm
    b. Voltage: 0.65 kV
    c. Capacitance 25 uF
    d. Optimal time constant between 8 and 9.5
9. Quickly remove the cells from the cuvette with 1 mL of CYE and transfer to a tube with an additional 1.5 mL of CYE (9 mL de medio
10. Incubate at 32ºC for 4-5h with shaking at 300 rpm for recovery. A 2-3 h incubation may suffice for phage attachment plasmids or replicative plasmids, but for homologous recombination with sequences less than 500 bp an overnight recovery may be necessary.

11. Plate cells on selective medium as follows
    a. For attachment or replicative plasmids, plate 100 uL directly and 100 uL of a 1/10 dilution
    b. For homologous recombination and genomic DNa transformations, plate 100 to 150 uL directly. Concentrate the rest and plate on two plates.
12. Incubate plates at 32ºC for 5 to 6 days to obtain single colonies.